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Evaluating categories of resistance in soybean genotypes from the United States and Brazil to *Aphis glycines* (Hemiptera: Aphididae)

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Evaluating categories of resistance in soybean genotypes from the United States and Brazil to *Aphis glycines* (Hemiptera: Aphididae)

Edson L. L. Baldin¹, Lia Marchi-Werle², Luiz E. R. Pannuti^{1,*}, André L. Lourenção³, Tiffany M. Heng-Moss², and Thomas E. Hunt²

Abstract

Aphis glycines Matsumura (Hemiptera: Aphididae), the soybean aphid, has become an important pest of soybeans, leading to significant yield losses in the United States. Host plant resistance is a viable alternative for managing *A. glycines*. The objectives of this study were to identify and categorize sources of resistance in soybean to *A. glycines* on genotypes from the United States and Brazil. An antixenosis assay was initially conducted with 8 genotypes to evaluate attractiveness to *A. glycines*. The selected soybean genotypes were further evaluated in a colonization assay to investigate the resistance of the genotypes at V1 (fully developed leaves at unifoliate node, 1st trifoliate leaf unrolled) and V3 (fully developed leaf at 2nd trifoliate node, 3rd trifoliate leaf unrolled) stages. An antibiosis assay was also conducted, in which multiple biological parameters of *A. glycines* were recorded. In the antixenosis assay, PI 200538, IAC 24, and IAC 17 genotypes were least attractive to adults of *A. glycines*, indicating moderate levels of antixenosis. The colonization assay showed that genotypes infested at the V3 stage had greater resistance when compared with the respective plants infested at the V1 stage. In addition, high levels of antibiosis to *A. glycines* were found in UX 2569-159, PI 200538, and PI 243540 genotypes. The identification of soybeans with resistance to *A. glycines* is of importance for the integrated pest management of this insect pest in the United States. Moreover, this research represents the first report on potential sources of resistance to *A. glycines* in soybeans from Brazil.

Key Words: host plant resistance; soybean aphid; antixenosis; antibiosis

Resumo

O pulgão-da-soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), tornou-se uma importante praga da soja, levando a significativas perdas de produção nos Estados Unidos da América (EUA). A resistência de plantas é considerada uma alternativa viável para o manejo de *A. glycines*. Os objetivos deste estudo foram identificar e categorizar fontes de resistência em soja a *A. glycines*, avaliando genótipos dessa leguminosa do Brasil e EUA. Um ensaio de antixenose foi conduzido inicialmente com oito genótipos de soja a fim de avaliar a atratividade de *A. glycines*. Os genótipos selecionados foram posteriormente avaliados em um ensaio de colonização, visando verificar sua resistência nos estádios V1 (folhas do primeiro nó totalmente desenvolvidas; primeiro trifólio aberto) e V3 (folhas do segundo trifólio totalmente desenvolvidas; folhas do terceiro trifólio abertas). Um ensaio de antibiose também foi realizado, avaliando-se diversos parâmetros biológicos de *A. glycines*. No ensaio de antixenose, os genótipos PI 200538, IAC 24 e IAC 17 foram menos atrativos a adultos de *A. glycines*, indicando níveis moderados de antixenose. O ensaio de colonização mostrou que genótipos de soja infestados no estágio V3 apresentam maior resistência em comparação com plantas infestadas no estágio V1. Em adição, este estudo constatou altos níveis de antibiose para *A. glycines* nos genótipos UX 2569-159, PI 200538 e PI 243540. A identificação de genótipos de soja com resistência a *A. glycines* é importante para os programas de manejo integrado de pragas (MIP) nos EUA. Em adição, esta pesquisa apresenta o primeiro registro de genótipos de soja brasileiros como potenciais fontes de resistência ao pulgão-da-soja.

Palavras Chave: resistência de plantas; pulgão-da-soja; antixenose; antibiose

Aphis glycines Matsumura (Hemiptera: Aphididae), the soybean aphid, was first documented in North America in 2000 and has spread through the major soybean areas in the United States and Canada (Ragsdale et al. 2004, 2011; Venette & Ragsdale 2004). Native to Asia, *A. glycines* has become the primary pest of soybeans in the eastern and mid-western portions of the United States. The feeding injury by *A. glycines* may result in yield losses that surpass 40% (Rice et al. 2005; Ragsdale et al. 2007; Kim et al. 2014). To date, *A. glycines* has not

been detected in Brazil (Hirose & Moscardi 2012); however, soybean is grown on all continents and has intense international trade, favoring soybean aphid's invasion around the world (Hoffmann-Campo et al. 2003). Dozens of species of insects that occur in soybeans in other regions of the world are potential pests of soybean in Brazil, including *A. glycines* (Hirose & Moscardi 2012).

The infestation pattern of *A. glycines* varies according to phenological stages of soybeans (McCornack et al. 2008). During the vegetative

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period of soybean, the insect commonly feeds on the newly emerged trifoliates, primarily on the upper portion of these leaves. As aphid density increases and soybeans mature, the insects colonize the lower canopy and eventually other parts of the soybean, including petiole, stem, and pods (Ragsdale et al. 2004). Feeding by *A. glycines* can cause soybean stunting, which ultimately impacts the weight and number seeds (Li et al. 2004; Beckendorf et al. 2008). The accumulation of honeydew during phloem sap ingestion by *A. glycines* favors the growth of dark sooty mold and may reduce photosynthetic rates (Macedo et al. 2003; Tilmon et al. 2011). *Aphis glycines* may also damage soybeans by transmitting certain viruses, such as alfalfa mosaic virus, soybean dwarf virus, and soybean mosaic virus (Sama et al. 1974; Iwaki et al. 1980). In addition to severe yield loss, the transmission of these viruses also impacts soybean seed quality (Hill et al. 2001; Clark & Perry 2002).

In an attempt to minimize the damage caused by *A. glycines*, soybean growers mostly rely on pest monitoring and the use of chemical control (foliar application or seed treatment) (Song & Swinton 2009; Ragsdale et al. 2011; Tilmon et al. 2011). While insecticides reduce *A. glycines* populations, the intense use of these chemicals has a negative impact on natural enemies (Kraiss & Cullen 2008; Ohnesorg et al. 2009) and increases the risk of insecticide resistance (Chandrasena et al. 2011).

Host plant resistance is a valuable tactic for managing *A. glycines*. Although the commercial availability of aphid-resistant soybean is still limited, intensive research has been conducted. Studies have identified soybean genotypes expressing various types of resistance to *A. glycines*, including antixenosis and/or antibiosis and tolerance (Hill et al. 2004, 2006a,b, 2009; Li et al. 2004; Mensah et al. 2005; Diaz-Montano et al. 2006, 2007; Mian et al. 2008a; Crompton & Ode 2010; Pierson et al. 2010, 2011; Hesler et al. 2012; Marchi 2012; Prochaska et al. 2013). Recently, multiple resistance genes were identified and mapped on different chromosomes of several *A. glycines* resistant soybean genotypes. For example, *Rag1* gene identified in 'Dowling', *Rag* in 'Jackson' (Li et al. 2007), and *Rag1c* in PI 567541B (Zhang et al. 2009) were mapped to a region in chromosome 7 of soybeans. The *Rag2* gene in PI 243540 (Mian et al. 2008b) and PI 200538 (Hill et al. 2009) and *Rag4* present in PI 567541B (Zhang et al. 2009) were mapped to chromosome 13, as well as *Rag3* in PI 567543C and *Rag3b* in PI 567537 to the same region (Zhang et al. 2010; 2013). These accessions from United States Department of Agriculture soybean germplasm were classified as sources of resistance to *A. glycines*, known to possess antixenosis and antibiosis (Li et al. 2007; Jun et al. 2012). Further, a resistance gene in PI 567301B was also mapped to the same position of *Rag2* gene of PI 200538 and PI 243540, but the resistance reported in PI 567301B is antixenosis rather than the common antibiotic effect of this gene (Jun et al. 2012).

After the identification of several *Rag* genes, *A. glycines* populations were categorized into biotypes according to their response to these genes. Kim et al. (2008) designated biotype 2 from a colony of *A. glycines* that survived on soybeans Dowling and Jackson (presence of *Rag1*), whereas biotype 1 colonies were considered susceptible to the aforementioned genotypes. However, genotypes PI 200538, PI 567541B, and PI 567597C are resistant to both *A. glycines* biotype 1 and biotype 2. Hill et al. (2010) named virulent populations of *A. glycines* that colonized soybean plants with *Rag1c*, *Rag2*, *Rag3*, and *Rag4* genes as biotype 3. However, biotype 3 colonies were unable to infest *Rag1* gene in Dowling (Hill et al. 2010). Recently, biotype 4 was identified in North America. This virulent population overcame *Rag1* and *Rag2* genes, including the stacked material, which contained a combination of these 2 genes (Alt & Ryan-Mahmutagic 2013). The existence of biotypes jeopardizes the development and effectiveness of resistant soybean genotypes as well as the durability assigned to this management strategy (Michel et al. 2011). Therefore, finding novel sources of resistance to *A. glycines* is necessary to maintain an effective management of this pest.

Although no reports have suggested the presence of *A. glycines* in Brazil, there is an imminent risk for its introduction when considering the extension of soybean growing regions and high adaptability of this insect. The evaluation of Brazilian soybean genotypes with resistance to other hemipteran pests (Valle & Lourenção 2002; Silva et al. 2012, 2013; Valle et al. 2012) may indicate reasonable candidates for resistance to *A. glycines*. Therefore, the present study evaluated the attractiveness, colonization, and performance of *A. glycines* on various soybean genotypes from the United States and Brazil with the objective of identifying categories of resistance.

Materials and Methods

REARING AND MAINTENANCE OF *A. GLYCINES*

Individuals of *A. glycines* were initially collected in commercial soybean fields near the University of Nebraska, Northeast Research and Extension Center, Haskell Agricultural Laboratory in Concord, Nebraska (42.3841667°N, 96.9891667°W) during the growing season of 2011. The insects were maintained on soybean KS4202 (V2–V6 stages) in a growth chamber (23 ± 2 °C and 16:8 h L:D photoperiod), and were progenies of a Nebraska isolate (biotype 1).

PLANT MATERIAL

Eight soybean genotypes were evaluated for *A. glycines* resistance. The genotypes evaluated were: KS4202 (tolerant), SD01-76R (susceptible), PI 200538 (reported resistance), PI 243540 (reported resistance), IAC 17 (promising; unknown resistance), IAC 19 (promising; unknown resistance), IAC 24 (promising; unknown resistance), and UX2569-159 (promising; possible resistance) (Table 1). The genotypes were selected based upon parental resistance, economic and scientific interests, and seed availability.

ANTIXENOSIS ASSAY

Due to the great variability in choice assays and in order to perform methodological adjustments, a preliminary assay using the genotype KS4202 was performed. The assay described by Diaz-Montano et al. (2006) utilized a circular piece of cardboard (with the projection to the base of each plant) on the soil of the pot to facilitate the movement of insects and assess the density of insects in each replication. Mobility and behavior of *A. glycines* during the day, and the number of insects per plant (%) within each replication were recorded.

Seven KS4202 seeds were sown in potting media (34% peat, 31% perlite, 31% vermiculite, and 4% soil mix) in 15 L round plastic pots. Soybeans were grown in a greenhouse under 400 W high-intensity lamps, 23 ± 3 °C, 60 ± 10% RH, and a photoperiod of 16:8 h L:D. Seeds were arranged in circle, equidistant, and near the margin of the pot. When plants reached the V1 stage (Fehr & Caviness 1977), 100 adults of *A. glycines* (starved for 1 h) were released at the base of the cardboard center (Diaz-Montano et al. 2006). The number of insects attracted by each plant was visually assessed after 1, 2, 3, 6, and 24 h of release. Each pot represented 1 replication (5 in total) arranged in a randomized block design.

Based on the results of the preliminary antixenosis assay (Table 2), a definitive antixenosis assay was performed with the soybean genotypes previously described. Subsequently, 140 apterous adult aphids were released (following 1 h starvation) per pot (20 individuals per plant). The number of insects attracted by genotype was evaluated after 24 h. In this assay, each pot represented a replication (20 in total) arranged in a randomized block design.

Table 1. Soybean genotypes evaluated for *Aphis glycines* resistance.

Genotype	Pedigree and description	Origin	References
KS4202	F4 plant selection from KS4694 × C1842. Tolerant to <i>A. glycines</i>	United States	Prochaska et al. 2013; Marchi-Werle et al. 2014
SD01-76R	(Stride × Resnik RR) × Stride Susceptible (without <i>Rag2</i> gene)	United States	Chiozza et al. 2010; Marchi-Werle et al. 2014
PI 200538	Resistant to <i>A. glycines</i> (<i>Rag2</i> gene)	Japan	Hill et al. 2009; Kim et al. 2010
PI 243540	Resistant to <i>A. glycines</i> (<i>Rag2</i> gene)	Japan	Mian et al. 2008b; Kang et al. 2008
IAC 17	D-72-9601-1 × IAC 8. Resistant to stink bugs and whiteflies	Brazil	Valle & Lourenção 2002; Silva et al. 2012; Valle et al. 2012
IAC 19	D-72-9601-1 × IAC 8. Resistant to stink bugs and whiteflies	Brazil	Valle & Lourenção 2002; Silva et al. 2012, 2013; Valle et al. 2012
IAC 24	IAC80-1177 × IAC83-288. Resistant to stink bugs and whiteflies	Brazil	Valle & Lourenção 2002; Silva et al. 2012, 2013; Valle et al. 2012
UX2569-159	U06-607094 × UX2324-34. Indications of resistance to <i>A. glycines</i>	United States	Preliminary tests

Table 2. Number (mean ± SE) of *Aphis glycines* individuals and damage level (mean ± SE) on 7 soybean genotypes (V1 and V3 stages) at 7, 14, and 21 d after soybean aphid infestation (DAI) (23 ± 3 °C; 60 ± 10% RH; 16:8 h L:D photoperiod).

Genotype	Aphid infestation ^a		Damage level ^b	
	7 DAI		7 DAI ^c	
	V1 stage	V3 stage	V1 stage	V3 stage
PI 200538	33.8 ± 4.9 b	9.0 ± 1.5 b	1.0 ± 0.0	1.0 ± 0.0
PI 243540	60.3 ± 10.3 b	28.3 ± 5.4 b	1.0 ± 0.0	1.0 ± 0.0
SD01-76R	109.8 ± 8.6 a	89.4 ± 6.6 a	1.0 ± 0.0	1.0 ± 0.0
IAC 19	111.4 ± 8.7 a	82.8 ± 15.4 a	1.0 ± 0.0	1.0 ± 0.0
IAC 17	120.2 ± 18.5 a	71.0 ± 11.3 a	1.0 ± 0.0	1.0 ± 0.0
IAC 24	120.5 ± 11.6 a	91.7 ± 7.1 a	1.0 ± 0.0	1.0 ± 0.0
KS4202	128.0 ± 15.7 a	101.3 ± 8.4 a	1.0 ± 0.0	1.0 ± 0.0
<i>P</i> (G)	<0.0001	—	—	—
<i>P</i> (V)	<0.0001	—	—	—
<i>P</i> (G × V)	0.9329	—	—	—
Genotype	14 DAI		14 DAI ^c	
	V1 stage	V3 stage	V1 stage	V3 stage
PI 200538	40.3 ± 6.7 cA	10.1 ± 5.1 cB	1.0 ± 0.0	1.0 ± 0.0
PI 243540	185.2 ± 33.6 bA	88.4 ± 34.9 bB	1.0 ± 0.0	1.0 ± 0.0
SD01-76R	497.8 ± 127.1 aA	324.5 ± 49.6 aA	1.0 ± 0.0	1.4 ± 0.2
IAC 19	565.6 ± 95.0 aA	459.0 ± 115.3 aA	1.0 ± 0.0	1.4 ± 0.2
IAC 17	551.7 ± 155.6 aA	364.5 ± 82.9 aA	1.0 ± 0.0	1.0 ± 0.0
IAC 24	577.8 ± 95.8 aA	460.8 ± 101.6 aA	1.1 ± 0.1	1.0 ± 0.0
KS4202	640.1 ± 152.4 aA	328.5 ± 31.1 aA	1.1 ± 0.1	1.0 ± 0.0
<i>P</i> (G)	<0.0001	—	—	—
<i>P</i> (V)	<0.0001	—	—	—
<i>P</i> (G × V)	0.0345	—	—	—
Genotype	21 DAI		21 DAI	
	V1 stage	V3 stage	V1 stage	V3 stage
PI 200538	90.5 ± 25.8 cA	202.5 ± 132.2 cA	1.0 ± 0.0 dA	1.0 ± 0.0 dA
PI 243540	1,266.4 ± 406.1 cA	489.2 ± 207.2 cB	1.1 ± 0.1 dA	1.0 ± 0.0 dA
IAC 19	3,539.6 ± 439.4 bA	4,085.6 ± 281.9 bA	2.1 ± 0.2 bB	2.8 ± 0.2 aA
IAC 17	4,260.0 ± 567.8 abA	4,180.0 ± 860.4 abA	2.0 ± 0.3 bcA	1.3 ± 0.2 bcB
KS4202	4,948.9 ± 573.6 aA	6,073.3 ± 529.4 aA	1.8 ± 0.2 bcA	1.2 ± 0.2 bcB
IAC 24	5,158.7 ± 456.2 aA	5,160.0 ± 400.7 aA	2.3 ± 0.3 aA	1.5 ± 0.2 bB
SD01-76R	5,306.2 ± 639.1 aA	3,584.4 ± 240.7 aB	1.6 ± 0.3 cA	1.5 ± 0.3 cA
<i>P</i> (G)	<0.0001	—	<0.0001	—
<i>P</i> (V)	0.6349	—	0.0420	—
<i>P</i> (G × V)	0.0453	—	0.0019	—

^aMeans followed by the same lower case letter in the column or upper case letter in the row do not differ by Fisher's LSD test ($P > 0.05$).^bDamage level scale; 1 = ≤10% leaf area with yellowing; 2 = 11–30%; 3 = 31–50%; 4 = 51–75%, and 5 = >75% leaf area with yellowing or tissue death.^cNo statistical analysis was performed for the period.

COLONIZATION ASSAY

The distinction between the mechanisms of antibiosis and antixenosis requires the observation of several biological parameters of the insect and can be difficult to distinguish depending on its size and age (Smith 2005). Moreover, the susceptibility of the genotype may vary depending on phenological stage (Painter 1951). The genotypes selected for this study were: SD01-76R, KS4202, IAC 17, IAC 19, IAC 24, PI 243540, and PI 200538. The colonization assay was also performed in a greenhouse as described previously. The experimental design was completely randomized, with a 2×7 factorial treatment arrangement, with 2 phenological stages (V1 and V3 stages), 7 genotypes, and 10 replications. Three seeds of each genotype were planted in potting media in 15 cm diameter round plastic pots at a depth of approximately 3 cm. Seedlings were thinned to 1 plant per pot after germination. To ensure that aphid infestation occurred simultaneously for both V1 and V3 stages, planting dates were staggered. Each experimental unit was infested with 10 apterous adult aphids, which were confined in individualized tubular cages (15 cm in diameter by 61 cm in height) constructed of transparent plastic (Makrolon Tuffak Lexan) covered with organdy cloth.

Three evaluations were performed, 7, 14, and 21 d after the initial infestation (DAI), by visually counting the number of aphids present on the plants. Cumulative aphid-day (CAD) was also determined based on weekly evaluations. According to Hanafi et al. (1989), CAD is the most efficient way to measure the total pressure of aphids on plants over time. Aphids-days (AD) can be calculated using the equation: $AD = [(N1 + N2) / 2] \times T$, where N1 is the number of aphids per plant in the previous sampling, N2 is the number of aphids per plant in the following sampling, and T is the number of days between the 2 sampling dates. The final value, CAD (cumulative aphid-days), is obtained by summing the values of AD. Plants were also scored for *A. glycines* damage (Fig. 1), using a scale of 1 to 5, where a rating of 1 = $\leq 10\%$ leaf area with yellowing; 2 = 11–30%; 3 = 31–50%; 4 = 51–75%, and 5 = $> 76\%$ leaf area with yellowing or tissue death (Pierson et al. 2010; Marchi-Werle et al. 2014).

ANTIBIOSIS ASSAY

The colonization assay suggested the occurrence of antibiosis in PI 243540 and PI 200538 genotypes, where CAD was significantly lowest. In this case, the expression of isolated antibiosis or antibiosis in combination with antixenosis could not be dismissed (Smith 2005) because some parameters (e.g., insect feeding) were not measured. Thus, to better characterize the occurrence of antibiosis in the selected genotypes, a clip-cage assay was performed. The genotype UX2569-159 was also included because it had shown indications of antibiosis in a previous screening study (data not shown). IAC 24 was excluded due to a limited availability of seeds.

Clip-cages were constructed with double sided 2.54×2.54 cm foam mounting squares (3M Scotch, Saint Paul, Minnesota), with an inner circular area of 1.2 cm^2 . Cages were placed on leaves and covered with organdy fabric once aphids had been introduced. Plants of the selected genotypes were grown to V1 stage as described in the colonization assay. In order to avoid pre-conditioning (Panda & Khush 1995), 10 apterous adult female aphids were placed separately inside clip-cages on 5 leaflets of plants of each genotype. After 24 h, adult females were removed and resulting nymphs were maintained on the respective leaves for 4 d. On the 5th day, 30 nymphs were placed in isolation inside each clip-cage on leaflets from a different set of plants of the respective genotypes. Each nymph corresponded to a replication (30 per genotype) in a completely randomized design. The following parameters were recorded daily until the death of the last adult female: total number of nymphs produced, number of nymphs per adult, number of nymphs at 7 and 10 d after birth of the first nymph, length of pre-reproductive and reproductive phase, adult longevity, life cycle, and mortality rate at 5, 7, and 10 DAI.

STATISTICAL ANALYSES

CAD, damage ratings, aphid number, and biological parameters of *A. glycines* were analyzed by a generalized mixed model (PROC GLIMMIX, SAS version 9.2; SAS Institute 2008,). Normality assumption was verified by the Shapiro-Wilk test and homoscedasticity by Levene's test (Winer et al. 1991); data were normalized by log transformation when necessary. Means were separated when *F* tests were significant ($P \leq 0.05$) using Fisher LSD procedures.

Results

ANTIXENOSIS ASSAY

In the preliminary antixenosis assay, *A. glycines* showed similar attraction ($F = 1.1$; $df = 6$; $P = 0.39$) to KS4202 plants, regardless of the planting arrangements in the pot (Fig. 2). In total, 71.2% remained active on the plants, whereas 28.8% of the aphids released in the preliminary assay were not found. For the antixenosis assay with different soybean genotypes, PI 200538 (8.6 aphids per plant), IAC 24 (9.2), and IAC 17 (9.6) had significantly fewer aphids per plant ($F = 1.7$; $df = 6$; $P = 0.01$) than SD01-76R (24.4), the most attractive genotype (Fig. 3).

COLONIZATION ASSAY

Significant differences were observed for soybean genotypes in all assessments, for both infestation stages (V1 and V3) (Table 2). At 7 days



Fig. 1. Damage level scale (1 to 5); feeding damage caused by *Aphis glycines* on soybean leaves.

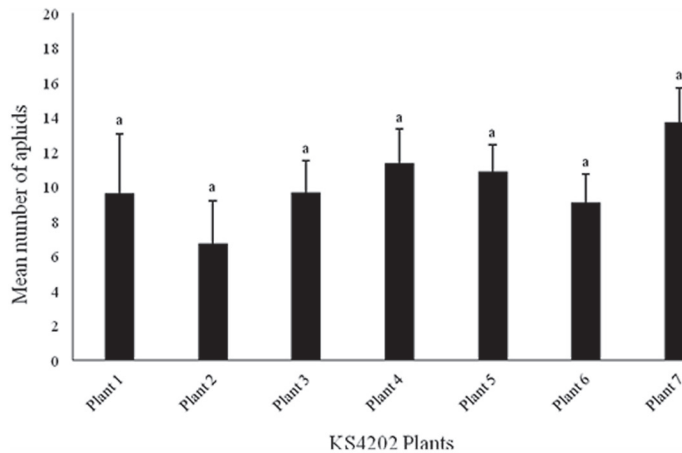


Fig. 2. Number (mean \pm SE) of *Aphis glycines* individuals on KS4202 plants 24 h after infestation (23 ± 3 °C; $60 \pm 10\%$ RH; 16:8 h L:D photoperiod). Means with the same lower case letter do not differ by Fisher's LSD test ($P > 0.05$). ($F = 1.09$; $df = 6$; $P = 0.3897$).

after infestation (DAI), PI 200538 and PI 243540 showed the lowest number of aphids at both the V1 ($F = 3.6$; $df = 6$; $P < 0.0001$) and V3 ($F = 3.1$; $df = 6$; $P < 0.0001$) stages, differing from the remaining genotypes. The interaction of genotype \times growth stages was not significant at 7 DAI. All genotypes showed damage levels equal to 1.0 ($\leq 10\%$ of leaf area with yellowing) during both infestation stages (Fig. 1).

At 14 DAI, PI 200538 and PI 243540 maintained the lowest aphid populations, differing from the other genotypes at the V1 ($F = 7.4$; $df = 6$; $P < 0.0001$) and V3 stages ($F = 6.4$; $df = 6$; $P < 0.0001$) (Table 2). Similarly to the evaluation at 7 DAI, aphid populations were also reduced when infestations occurred during the V3 stage. The interaction genotype \times growth stages was significant ($F = 5.8$; $df = 6$; $P = 0.03$) for PI 200538 and PI 243540, indicating that the phenological stage when plants were infested had an effect on *A. glycines* colonization. Damage ratings ranged from 1.0 to 1.1 (V1) and 1.0 to 1.4 (V3), similar to observations recorded at 7 DAI.

At 21 DAI, PI 200538 and PI 243540 also showed the lowest aphid populations (Table 2), differing from genotypes at the V1 ($F = 4.5$; $df = 6$; $P < 0.0001$) and V3 ($F = 3.9$; $df = 6$; $P < 0.0001$) stages. The interaction genotype \times growth stage was significant ($F = 7.8$; $df = 6$; $P = 0.04$) for both PI 243540 and SD01-76R, indicating a reduction in aphid popula-

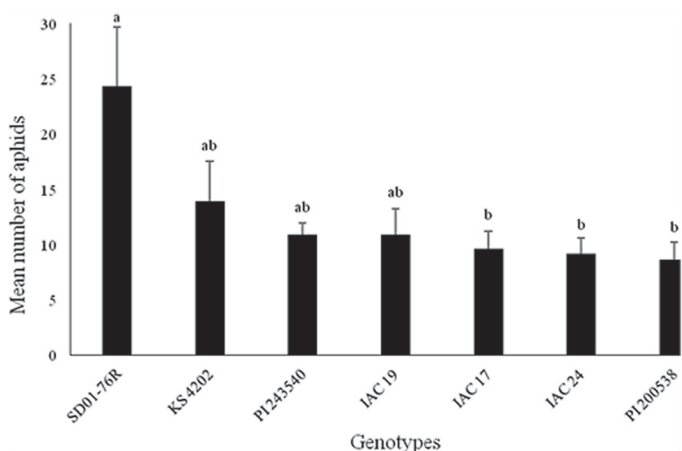


Fig. 3. Number (mean \pm SE) of *Aphis glycines* individuals on 7 soybean genotypes 24 h after infestation (23 ± 3 °C; $60 \pm 10\%$ RH; 16:8 h L:D photoperiod). Means with the same lower case letter do not differ by Fisher's LSD test ($P > 0.05$). ($F = 1.74$; $df = 6$; $P = 0.0110$).

tions when plants from the corresponding genotypes were infested at the V3 stage. The IAC genotypes analyzed in this study showed the highest damage ratings (2.0 to 2.25) when infested at the V1 stage (11–30% of leaf yellowing or chlorosis). Although IAC 19 supported a relatively intermediate aphid population, it had a higher damage rating (2.8) at the V3 stage than all other genotypes. Conversely, the damage ratings for the remaining infested genotypes at the V3 stage were consistently lower than those infested at the V1 stage.

When infested at the V1 stage, PI 243540 and PI 200538 had a CAD (cumulative aphid-days) of 246.0 and 153.3 aphid-days, respectively, whereas KS4202 accumulated 483.0 aphid-days (Fig. 4). At 14 DAI, the PI genotypes had CAD values between 153.3 and 246.0, whereas KS4202 (up to 3,000) and the remaining genotypes ranged from 2,500 to 3,000 CAD. At 21 DAI, PI 200538 and PI 243540 CAD still had the lowest CAD values (717.4 and 5,940 respectively), when SD01-76R, KS4202, and IAC 24 had accumulated over 20,000 CAD (Fig. 4). For plants infested at the V3 stage (Fig. 4), we observed similar performance for all genotypes in all 3 evaluations; however, CAD values were lower in comparison with those obtained with plants infested during the V1 stage.

ANTIBIOSIS ASSAY

Adult females of *A. glycines* that fed on UX2569-159 produced significantly fewer nymphs (2.1 nymphs) ($F = 19.4$; $df = 4$; $P < 0.0001$) than

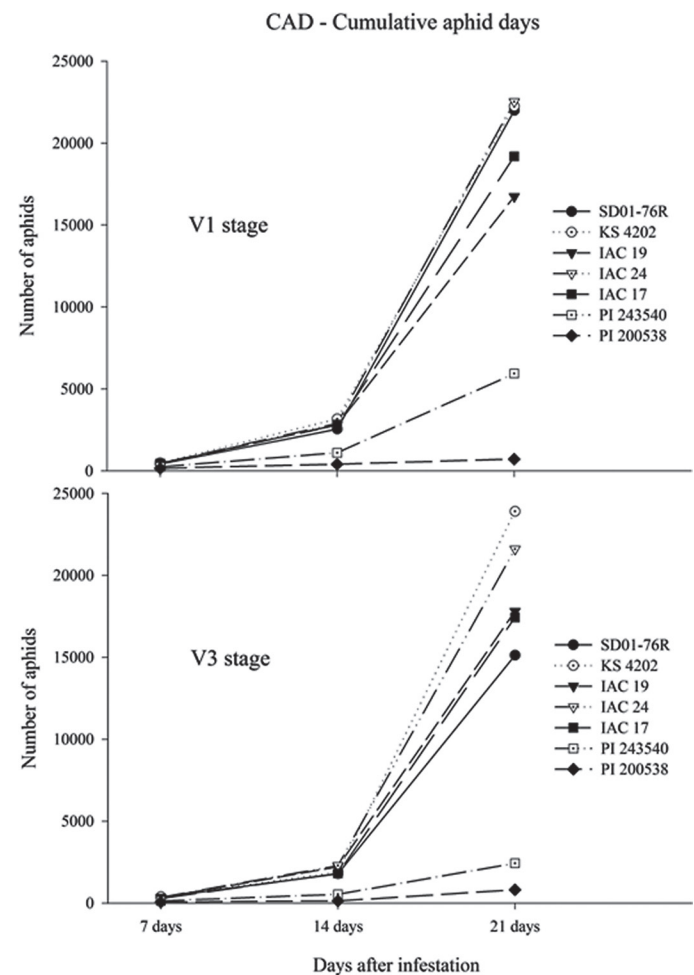


Fig. 4. Cumulative aphid-days (CAD) for soybean genotypes infested with *Aphis glycines* at V1 and V3 stages (23 ± 3 °C; $60 \pm 10\%$ RH; 16:8 h L:D photoperiod).

those that fed on IAC 17 (17.2), IAC 19 (19.3), KS4202 (20.4), and SD01-76R (21.1) (Table 3). Insects confined on PI 243540 and PI 200538 did not produce nymphs as the adult/reproductive phase was not reached. There were no significant differences within these genotypes for the number of nymphs produced per adult per day (Table 3). There were statistical differences for total nymphs produced at day 7 ($F = 3.8$; $df = 4$; $P = 0.004$) and day 10 ($F = 5.8$; $df = 4$; $P = 0.0001$) after the emergence of the first nymph. UX2569-159 had the lowest average (7.7 nymphs), differing from KS4202 (days 7 and 10) and SD01-76R (day 10).

The pre-reproductive phase was longer ($F = 4.8$; $df = 4$; $P = 0.0009$) in UX2569-159 (8.3 d) and IAC 17 (7.8 d) when compared with all other genotypes. Aphids on UX2569-159 showed a shorter reproductive phase (4.7 d) ($F = 12.3$; $df = 4$; $P < 0.0001$) than those on SD01-76R and KS4202 (10.2 and 9.2 d, respectively) (Table 3). Insects reared on UX2569-159 had shorter longevity (5 d) ($F = 7.5$; $df = 4$; $P = 0.0001$) and total cycle (7.8 d) ($F = 41.1$; $df = 6$; $P < 0.0001$) than those reared on SD01-76R (11.1 and 16.8 d, respectively) and KS4202 (10.3 and 16.0 d, respectively) (Table 3).

Five days after nymph introduction, nymphs of *A. glycines* feeding on PI 243540, PI 200538, and UX2569-159 had the highest mortality rates (93.3, 86.7, and 54.5%, respectively) (Fig. 5). After 7 d, nymphs on PI 243540 reached 100% mortality, whereas nymphs on PI 200538 and UX2569-159 had slightly lower mortality rates (93.3 and 60.0%, respectively). On day 10, the mortality rates on PI 200538 and UX2569-159 were 100 and 80%, respectively. Mortality on IAC 17 and IAC 19 did not exceed 10%, whereas mortality on KS4202 and SD01-76R genotypes was null up to 10 d after infestation (Fig. 5).

Discussion

Aphids remained active (~70%) in both antixenosis assays. The absence of statistical differences in the preliminary assay (conducted with KS4202) indicated that the position of the plants around the arena did not impact *A. glycines* host selection. Diaz-Montano et al. (2006) also used this method to characterize antixenosis in soybean genotypes to *A. glycines*, demonstrating the viability of this system. The definitive antixenosis assay showed that the genotypes PI 200538, IAC 24, and

IAC 17 harbored the lowest numbers of insects at 24 h, indicating that these genotypes have antixenotic effects on *A. glycines*. This resistance mechanism commonly affects insect behavior during host selection (Smith 2005).

According to Painter (1951), biophysical and biochemical properties present in plants may hinder the insect's recognition of a suitable host for feeding, oviposition, mating, or shelter. Antixenotic characteristics often limit or prevent feeding and oviposition due to the presence of repellents, absence of attractants, or imbalance between them. Other plant characteristics that may contribute to antixenosis are the epidermis, wax accumulation, and trichome type and density (Panda & Khush 1995; Smith 2005).

Diaz-Montano et al. (2006) also evaluated the resistance of soybean genotypes to *A. glycines* by using antixenosis assays. In their preliminary antixenosis assay, Pioneer 95B97 attracted fewer aphids when compared with the susceptible genotype at 24 h after infestation, indicating the presence of strong antixenosis. In a second assay, it was found that Jackson, Dowling, Palmetto, and K1639 were the least attractive in comparison with the susceptible genotype, also indicating the expression of antibiosis against *A. glycines* (Diaz-Montano et al. 2006). Moreover, *A. glycines* had a high attractiveness to KS4202 in Diaz-Montano et al.'s (2006) studies, which is consistent with our findings.

Although this is the first report of IAC's performance against *A. glycines*, there are numerous reports on the antixenotic and/or antibiogenic properties of these genotypes on whiteflies (*Bemisia tabaci* [Genadius]; Hemiptera: Aleyrodidae) and stink bugs (*Piezodorus guildinii* [Westwood]; Hemiptera: Pentatomidae) (Vieira et al. 2011; Silva et al. 2012, 2013). The antixenotic properties of PI 200538 to *A. glycines* in multiple-choice assays is documented for the first time; although the presence of antibiosis (related to *Rag2* gene) has been documented by Hill et al. (2009).

The genotypes PI 200538 and PI 243540 had the lowest aphid population levels in the colonization assay (up to 21 DAI), as well as lower CAD values in both V1 and V3 stages, confirming the presence of antibiosis during these stages (Mian et al. 2008b; Hill et al. 2009). Moreover, IAC 19 harbored a moderate aphid population in both plant stages, suggesting a moderate resistance to *A. glycines*.

Table 3. Means (\pm SE) of biological parameters of *Aphis glycines* on 7 soybean genotypes during antibiosis assay (23 ± 3 °C; $60 \pm 10\%$ RH; 16:8 h :D photoperiod).

Genotype	Total nymphs produced ^a	Nymphs produced per day	Total nymphs produced by day 7 ^a	Total nymphs produced by day 10 ^a
PI200538 ^b	—	—	—	—
PI243540 ^b	—	—	—	—
UX2569-159	2.1 \pm 1.1 b	1.5 \pm 0.2	7.6 \pm 1.2 b	7.7 \pm 1.2 c
IAC 17	17.2 \pm 3.7 a	1.8 \pm 0.3	11.1 \pm 2.5 ab	15.1 \pm 3.1 b
IAC 19	19.3 \pm 3.1 a	1.7 \pm 0.1	13.3 \pm 1.6 ab	17.2 \pm 2.4 b
KS4202	20.4 \pm 1.9 a	1.9 \pm 0.2	16.3 \pm 1.2 a	19.3 \pm 1.7 a
SD01-76R	21.1 \pm 3.2 a	1.7 \pm 0.1	13.7 \pm 1.4 ab	18.3 \pm 2.2 a
P	<0.0001	0.77	0.004	<0.0001

Genotype	Pre—reproductive stage (d) ^a	Reproductive stage (d) ^a	Longevity (d) ^a	Total cycle (d) ^a
PI200538 ^b	—	—	—	3.5 \pm 0.6 c
PI243540 ^b	—	—	—	2.7 \pm 0.4 c
UX2569-159	8.3 \pm 0.9 a	4.7 \pm 0.3 b	5.0 \pm 0.0 b	7.8 \pm 2.1 b
IAC 17	7.8 \pm 0.4 a	8.4 \pm 1.4 ab	9.0 \pm 1.4 ab	15.5 \pm 1.9 a
IAC 19	6.8 \pm 0.1 b	9.1 \pm 1.1 ab	9.8 \pm 1.3 ab	15.9 \pm 1.2 a
KS4202	6.7 \pm 0.1 b	9.2 \pm 0.8 a	10.3 \pm 1.1 a	16.0 \pm 1.0 a
SD01-76R	6.7 \pm 0.1 b	10.2 \pm 1.3 a	11.1 \pm 1.5 a	16.8 \pm 1.4 a
P	0.0009	<0.0001	<0.0001	<0.0001

^aMeans followed by the same lower case letter do not differ by Fisher's LSD test ($P > 0.05$).

^bGenotype inadequate to produce nymphs.

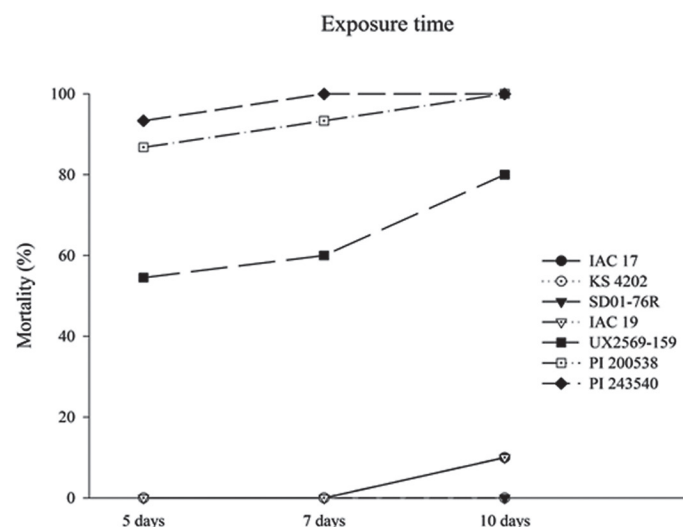


Fig. 5. Mortality (%) of *Aphis glycines* on 7 soybean genotypes at 5, 7, and 10 d after infestation (23 ± 3 °C; $60 \pm 10\%$ RH; 16:8 h L:D photoperiod).

The results of the interactions between genotypes and vegetative stages at 7 DAI suggested that aphid colonization on plants of each genotype was not affected by the stage of infestation. However, the results at 14 DAI (PI 200538 and PI243540) and 21 DAI (PI 243540 and SD01-76R) showed a reduction in colonization by *A. glycine* when plants were infested at the V3 stage. In general, this data indicates a greater susceptibility of these plants at the early stages of soybean growth. When comparing CAD values from V1 and V3 stages, there is a reasonable reduction in susceptibility as soybean plants are aging (Fig. 4 and Table 2). These results corroborate the findings of other authors (Pierson et al 2010; Marchi-Werle et al 2014), who reported that older (i.e., late vegetative to reproductive stage) soybeans are more resistant to *A. glycines* than plants in the early vegetative stage. Although IAC 17, IAC 19, and IAC 24 infested during the V1 stage showed moderate damage ratings (<3 on a scale from 1 to 5), it is possible that these damage ratings would be greater if the infestation was maintained for longer periods.

The low number of nymphs produced on UX2569-159 indicates the occurrence of antibiosis. Plants expressing this resistance mechanism may directly or indirectly impact the insect's reproduction (Smith & Clement 2012). High rates of mortality of juveniles, as well as reductions in size, weight, and fecundity of individuals are described as the most common effects of antibiosis (Panda & Khush 1995; Smith 2005).

Aphids that fed on PI 243540 and PI 200538 did not produce nymphs, as insect maturity was never reached. Li et al. (2004) reported high levels of antibiosis in Dowling, Jackson, and PI 200538, with significant reductions in fecundity and longevity and high mortality of *A. glycines* when compared with insects confined in the susceptible genotype. In addition, Diaz-Montano et al. (2006) found that adult aphids feeding on K-1639, Pioneer 95B97, Jackson, Dowling, and Palmetto produced fewer nymphs than those fed on KS4202 and Pioneer 93B15. According to these authors, the low reproduction rate of *A. glycines* indicated the expression of high levels of antibiosis. Antibiosis was also reported by Hesler et al. (2007), who documented a lower birth rate and reduced *A. glycines* population growth on Dowling, PI 71506, PI 230977, and G93-9223. Mian et al. (2008b) evaluated approximately 200 soybean genotypes and found strong antibiosis in 3 PI genotypes, especially PI 243540. In that study, PI 243540 had less than 2 nymphs produced at 14 d after initial infestation with 10 adults of *A. glycines*, which supports the findings of this study.

Aphis glycines had a prolonged pre-reproductive phase on UX2569-159 and IAC 17, suggesting the presence of deterrent compounds in these genotypes. Insects confined to genotypes expressing antibiosis and/or antixenosis may require more time to complete life stages due to inadequate nutrition in the host (Panda & Khush 1995). Aphids confined to UX2569-159 also had a shorter reproductive period and had a 50% reduction in longevity in comparison with aphids confined to SD01-76R and KS4202. Li et al. (2004) also found that *A. glycines* confined to susceptible genotype Pana lived 7 d longer than individuals on the resistant genotypes Dowling and Jackson. Regarding the total life cycle, our results indicate that the PI genotypes and UX2569-159 were highly resistant to *A. glycines*. In this case, the significant reduction was related to a high mortality rate in the early stages of development, which is a common characteristic of genotypes expressing antibiosis (Painter 1951).

The absence of nymphs in PI 243540 and PI 200538 indicated the occurrence of high levels of antibiosis against *A. glycines*. These results corroborate with studies by Mian et al. (2008b) and Hill et al. (2009). The lower production of nymphs, combined with reductions in reproductive phase, longevity, and total life cycle on UX2569-159 also indicated the occurrence of antibiosis. However, factors associated with this resistance require further attention.

The high mortality rates on the PI genotypes and UX2569-159 found on days 5 and 7 after aphid introduction confirmed the occurrence of antibiosis in these genotypes. The expression of antibiosis may vary by genetic characteristic of each line and can also be associated with the simultaneous occurrence of antixenosis (Painter 1951). Li et al. (2004) reported that 1st instar nymphs confined to leaves of genotypes Dowling and PI 200538 did not reach adulthood, but starvation did not fully explain the effects of antibiosis because aphids spent less time feeding on these plants. Therefore, Li et al. (2004) suggested that a combination of antixenosis and antibiosis occurred in Dowling and PI 200538. Considering the assays performed in this study, we report moderate levels of antixenosis in the IAC genotypes to *A. glycines*. This may explain the intermediate averages in the antibiosis assay (IAC 19), nymphs produced at 10 d (IAC 17 and IAC 19), and pre-reproductive phase (IAC 17) and the low aphid numbers in the antixenosis assay (IAC 17 and IAC 24), associated with low rates of mortality.

Research conducted to date has greatly contributed to increasing the knowledge in bio-ecological aspects and management options for *A. glycines*. However, several aspects involving different levels of susceptibility of genotypes and specific determination of economic thresholds for resistant genotypes should be addressed in future investigations. The results of this study have highlighted host plant resistance as a valuable strategy for managing *A. glycines* in the United States, especially the antibiosis checked in PI 200538, PI 243540, and UX2569-159. The genotypes from Agronomic Institute of Campinas (IAC), São Paulo, Brazil, showed some promising results, indicating the occurrence of moderate antixenosis to *A. glycines*. The comparison of these genotypes with North American genotypes is necessary and may serve as a basis for future breeding programs when considering the possibility of *A. glycines* invasion in Brazil or countries with a similar agroecosystem.

Acknowledgments

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